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Single-stranded nucleic acid-induced helical self-assembly of alkynylplatinum(II) terpyridyl complexes

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Single-stranded nucleic acids, which carry multiple negative charges in an aqueous medium at near neutral pH, are found to induce the aggregation and self-assembly of the positively charged alkynylplatinum(II) terpyridyl complexes via electrostatic binding of the platinum complexes to the single-stranded nucleic acids, as revealed by the appearance of new UV-vis absorption and emission bands upon addition of single-stranded nucleic acids to a buffer solution of the complex. Changes in the intensity and pattern of circular dichroism (CD) spectroscopy are also observed, many of which are consistent with the assembly of the platinum complexes into helical structures, via metal-metal and π - π stacking interactions. The induced spectroscopic property changes are found to depend on the structural properties of the nucleic acids.

helicity | noncovalent interactions | platinum complex

Nucleic acids are, in a sense, the most fundamental and important class of biomolecules in a living cell. Detection of nucleic acids, analysis of their sequence, structure, and the corresponding properties, as well as their self-replication, interactions with regulation factors, and the details of transcription and translation are among the major foci of numerous biochemical and biophysical research works (1, 2). Single-stranded nucleic acid sensing and characterization are therefore of great importance, which not only can help us to understand how the cell functions and to assist biological research, but also facilitate the development of new tools for disease diagnosis and treatment and new drug developments.

It has been known for many years that many of the square planar metal complexes arrange themselves into highly ordered extended linear chain or oligomeric structures in the solid state. Depending on the extent of the metal-metal and the ligand π - π stacking interactions, different colors could be observed (3, 4). Square planar platinum(II) complexes belong to a particularly interesting class of this type of complexes, as a result of their rich spectroscopic and luminescence properties revealed during the past few decades (3–16).

Recently, we have synthesized a number of platinum(II) polypyridyl alkynyl complexes that show interesting spectroscopic and luminescence properties (17–21). One particularly interesting observation is that solvents of different polarity and also polyacrylate could induce the aggregation of the positively charged platinum complexes in organic solvent mixtures (19, 21). As a result, remarkable spectroscopic property changes were observed. Because single-stranded nucleic acids carry multiple negatively charged phosphate functional groups, they are polyanions. It is therefore reasonable to envisage that single-stranded nucleic acids may have the possibility to induce the self-assembly of the square planar platinum(II) complexes in an aqueous environment. Here, we describe the synthesis and characterization of a water-soluble platinum(II) terpyridyl alkynyl complex, [Pt(tpy)C \equiv CC \equiv CCH₂OH]OTf (1) (Fig. 1), and its spectroscopic properties in the presence and absence of single-stranded

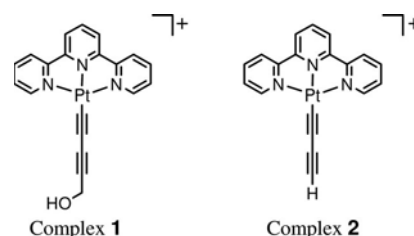


Fig. 1. Structure of the platinum(II) complexes.

nucleic acids. The interaction of single-stranded nucleic acids with a related [Pt(tpy)C \equiv CC \equiv CH]OTf (2) has also been explored by using UV-vis, emission, and circular dichroism (CD) spectroscopy.

Results and Discussion

Two metal complexes are used in the present investigation, namely complexes 1 and 2 (Fig. 1). Both metal complexes have certain properties that render them especially suitable for the self-assembly studies described in the present work. Both of them are square planar in geometry and contain a d⁸ platinum(II) center that is capable of participating in metal-metal interactions. They also contain an aromatic terpyridine ligand that can interact with each other by π - π stacking interactions.

Complex 2 is found to have rather limited solubility in aqueous media. Nevertheless, at dilute concentrations ($\approx 30 \mu\text{M}$), 2 completely dissolves in water to give a clear solution. To reduce the possible nonspecific hydrophobic interactions with the nucleic acid base, and also to improve the complex solubility in water, a hydroxymethyl group is introduced at the butadiynyl end to give complex 1 (Fig. 1). Solutions of 1 in water could be readily prepared, with concentrations of $\approx 0.45 \text{ mM}$ readily achieved.

A concentration-dependent UV-vis absorption study shows that Beer's law is not obeyed at wavelength $\geq 285 \text{ nm}$ at complex concentrations higher than $\approx 15 \mu\text{M}$ for complex 1, suggesting the formation of dimer or oligomer under these conditions (7, 8, 12, 16). A new absorption shoulder at ≈ 520 – 530 nm , which becomes more obvious in an aqueous medium of higher ionic strength (10 mM Tris-HCl/50 mM NaCl, pH 7.5), has been observed with increasing complex concentration and is ascribed

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Abbreviation: MMLCT, metal-metal-to-ligand charge transfer.

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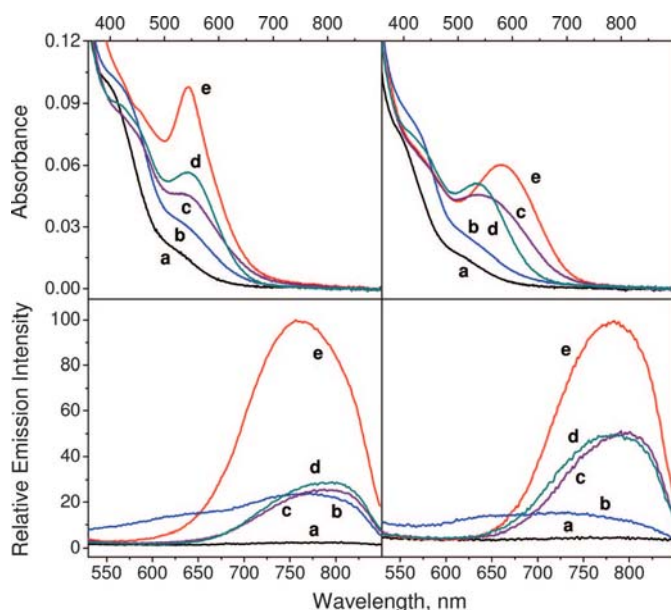


Fig. 2. UV-vis and emission spectra. (Upper) UV-vis absorption spectra of 30 μM of complexes **1** (Left) and **2** (Right) (line a), and their binding to 90 μM (base concentration) of poly(dA)₂₅ (line b), poly(dC)₂₅ (line c), poly(dG)₂₅ (line d), and poly(dT)₂₅ (line e), respectively. Medium used was 5 mM Tris-HCl/10 mM NaCl, pH 7.5. (Lower) Emission spectra of 30 μM of complexes **1** (Left) and **2** (Right) (line a), and their binding to 90 μM of poly(dA)₂₅ (line b), poly(dC)₂₅ (line c), poly(dG)₂₅ (line d), and poly(dT)₂₅ (line e), respectively. Medium used was 5 mM Tris-HCl/10 mM NaCl, pH 7.5. Spectra were not corrected for PMT response.

to a metal-metal-to-ligand charge transfer (MMLCT) transition. Electrospray-ionization mass spectrometry (ESI-MS) experiments show the presence of the monomer, dimer, and trimer at high concentrations of complex **1**. (Higher oligomers could not be observed in the ESI-MS experiments because their m/z values are beyond the detection range of our instrument.) In addition, a very weak emission band at ≈ 800 nm also starts to appear at ≈ 15 μM complex concentration, and its intensity becomes stronger with increasing complex concentrations, probably ascribed to an excited state of a triplet metal-metal-to-ligand charge transfer (³MMLCT) origin based on previous studies on related systems (17–21). Nevertheless, at a 30 μM complex concentration, which is used throughout the current investigation, the 800 nm background emission bands are very weak and just barely recognizable for both complexes.

Fig. 2 Upper shows the UV-vis absorption spectra of complexes **1** and **2**, and the corresponding spectral changes when mixed with various oligonucleotides, namely poly(dT)₂₅, poly(dC)₂₅, poly(dG)₂₅, and poly(dA)₂₅, respectively. In an aqueous solution with constant pH and ionic strength (5 mM Tris-HCl/10 mM NaCl, pH 7.5) at ambient temperature, remarkable absorption changes are observed. Depending on the sequence of the oligonucleotide, the absorption changes vary quite significantly. Whereas, for poly(dT)₂₅, new bands form at ≈ 544 nm and 581 nm for complexes **1** and **2**, respectively, no new band formation occurs and only weak absorbance changes are observed for poly(dA)₂₅. For poly(dC)₂₅ and poly(dG)₂₅, new band formations for complexes **1** and **2** with moderate intensity are observed.

We also observe that concomitant with the remarkable UV-vis absorption changes, for poly(dT)₂₅, poly(dC)₂₅, and poly(dG)₂₅, upon mixing of the platinum(II) complexes and the oligonucleotides together, new emission bands at ≈ 800 nm appear.

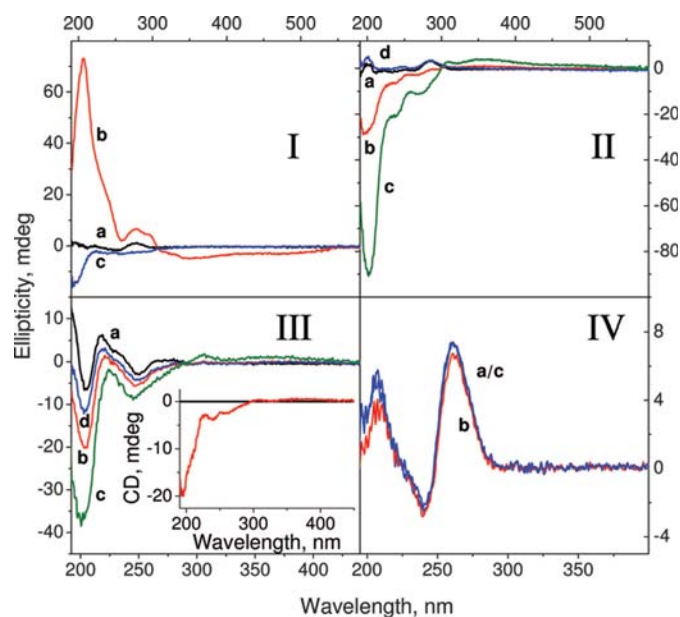


Fig. 3. CD spectra. (I) 90 μM of poly(dT)₂₅ (line a), and its binding with 30 μM of complexes **1** and **2** (lines b and c, respectively). (II) 90 μM of poly(dC)₂₅ (line a), and its binding with 30 and 45 μM of complex **1**, and 30 μM of complex **2** (lines b, c, and d, respectively). (III) 90 μM of poly(dA)₂₅ (line a), and its binding with 30 and 60 μM of complex **1**, and 30 μM of complex **2** (lines b, c, and d, respectively). (Inset) Spectrum equals line b minus line a. (IV) 90 μM of poly(dG)₂₅ (line a), and its binding with 30 μM of complexes **1** and **2** (lines b and c, respectively). Medium used was 5 mM Tris-HCl/10 mM NaCl, pH 7.5.

Fig. 2 Lower shows the emission spectra of complexes **1** and **2**, and the corresponding spectral changes of their mixtures with various oligonucleotides, respectively. At a 30 μM concentration, the complexes alone are barely emissive, whereas significant emission spectral changes for the various metal complex–oligonucleotide mixtures are observed. Depending on the sequence of the oligonucleotide, the intensity changes vary quite significantly. For poly(dT)₂₅, new intense emission bands are observed at ≈ 760 nm and 782 nm for the mixtures with complexes **1** and **2**, respectively. In contrast, very broad emission bands with no clear band maximum are observed for mixtures of complexes **1** and **2** with poly(dA)₂₅; the emission intensity is moderate for complex **1** and particularly weak for complex **2**. For the mixtures of complexes **1** and **2** with poly(dC)₂₅ and poly(dG)₂₅, new emission bands with band maximum at ≈ 800 nm and of moderate intensity are observed for both complexes.

On the basis of our previous work (17–21) and other related studies (3–16), the newly formed UV-vis bands of complexes **1** and **2** at longer wavelength are assigned as MMLCT transitions, as a result of the self-assembly of the complexes induced by the oligonucleotides through metal–metal and π – π interactions. The newly formed emission bands are attributed to MMLCT triplet emission. The induced self-assembly of the complex in an aqueous solution could likely be interpreted based on the structural properties of the complex and the nucleic acid. The structure of the complex is planar and it carries a positive charge. Nucleic acid on the other hand at near neutral pH carries multiple negative charges. Electrostatic interactions between the positive and negative charge bring the complex and the nucleic acid molecule into close proximity, i.e., binding of the complex molecules to the nucleic acid. As a result, the local concentration of the complex is increased. Equally important, the positive charge carried on the complex molecule is balanced out by the negative charge on the biopolymer, and hence the electrostatic repulsive force between the complex molecules is largely re-

moved. The complex could therefore easily self-assemble via metal...metal and π ... π interactions.

Fig. 3 shows the CD spectra of the four oligonucleotides studied and the corresponding spectral changes upon mixing with complexes **1** and **2**, respectively. The results clearly show that different oligonucleotide-metal complex mixtures give dramatically different CD spectra. When complex **1** is mixed with poly(dT)₂₅, a very broad negative band spanning from ≈ 305 to ≈ 550 nm of moderate intensity, a positive band between ≈ 258 and 305 nm of moderate intensity, and a very strong positive band between 190 and 258 nm are observed (Fig. 3I). In sharp contrast, when complex **1** is mixed with poly(dC)₂₅, especially at a higher complex concentration, a CD spectrum that shows a mirror image relationship with respect to that with poly(dT)₂₅ is observed (Fig. 3II).

The mixture of complex **1** with poly(dA)₂₅ shows comparatively smaller CD spectral changes. However, the spectra, especially with a higher complex concentration (60 μ M, Fig. 3III, curve c), look quite similar in shape to the CD spectra of the mixture of complex **1** with poly(dC)₂₅. If the CD signal of poly(dA)₂₅ is subtracted from the total CD spectrum, the general similarity becomes more evident (Fig. 3III Inset).

It is observed that for the solution mixtures of poly(dT)₂₅, poly(dC)₂₅, and poly(dA)₂₅ with complex **1**, an increase in the complex concentration from 30 to 45 μ M and 60 μ M while keeping the oligonucleotide concentration unchanged (90 μ M, base concentration) would give rise to a significant CD intensity enhancement, whereas the general shape of the CD spectra remains essentially unchanged (for example Fig. 3 II and III, curve c). A decrease in the complex concentration or an increase in the oligonucleotide concentration leads to decreased CD intensity, with the shape of the CD spectra again remains unchanged.

The CD signals of complex **2** when mixed with poly(dT)₂₅ and poly(dA)₂₅ are weaker than that of complex **1**. However, their general shape is also similar to the CD spectrum of the mixture of complex **1** and poly(dC)₂₅. Complex **2**, when mixed with poly(dC)₂₅, gives very small CD changes. It is interesting to note that neither complex **1** nor complex **2**, when mixed with poly(dG)₂₅, gives rise to any significant CD changes. In fact, there are almost no CD changes at all (Fig. 3IV), and very small CD spectral changes are observed even when the concentration of complex **1** is doubled.

The strong CD changes observed in the mixtures of metal complexes and oligonucleotides are believed to mainly originate from electronic transitions characteristic of the metal complexes. The induced CD signals at wavelengths beyond 300 nm are ascribed to that of the platinum complexes because nucleic acids do not show significant absorption in this region (22, 23), whereas complexes **1** and **2** show intense absorptions in the visible region. Even at wavelengths between 190 and 300 nm, where the nucleic acids absorb strongly, the strong induced CD signal changes are believed to mainly originate from electronic transitions of the metal complexes. This could be supported based on the following grounds. The maximum and minimum $\Delta\epsilon$ values (24, 25) for poly(dT) alone (in the range of +6 to -4, unit $M^{-1} cm^{-1}$), poly(dA)·poly(dT) duplex (in the range of +20 to -10), and poly(dA) (in the range of +16 to -22) are too small to account for the strong CD signals induced [calculated maximum or minimum $\Delta\epsilon$ values for complex **1** mixed with poly(dT)₂₅ (Fig. 3I, curve b) and poly(dA)₂₅ (Fig. 3III, curve b) are 246 and -68 $M^{-1} cm^{-1}$, respectively, assuming they are from the nucleic acids].[†] Moreover, the general shape, position, and

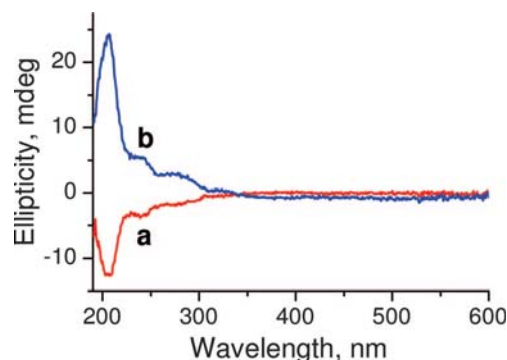


Fig. 4. CD spectra of 30 μ M of complex **1** binding to 90 μ M (carboxylate concentration) of poly(L-aspartate) (line a) and poly(L-glutamate) (line b), respectively. Medium used was 5 mM Tris-HCl/10 mM NaCl, pH 7.5.

intensity of the positive and negative bands of the CD spectra of the oligonucleotides, poly(dT)₂₅, poly(dC)₂₅, and poly(dA)₂₅, are quite different from each other, and do not match with the CD of the oligo-complex mixtures. Finally, the intensity dependence of the induced CD on the concentration of the metal complexes and oligonucleotides also suggests that the induced CD signals are associated with the metal complexes.

Further support comes from an independent study with poly(amino acids). When complex **1** is mixed with poly(L-glutamate) or poly(L-aspartate), UV-vis and emission studies show very good induced complex aggregation, as revealed from the appearance of the MMLCT absorption band at ≈ 550 nm and MMLCT emission band at ≈ 798 nm, and in addition, large induced CD signals are observed (Fig. 4) [poly(L-glutamate) and poly(L-aspartate) alone give only very weak CD signals, data not shown]. When complex **1** is mixed with poly(D-glutamate), the induced CD spectrum is found to be quite similar in shape to that of poly(L-aspartate), and is the exact mirror image of the CD obtained with poly(L-glutamate). More importantly, the general shape of the CD spectra matches quite well with that obtained with the oligonucleotides. Therefore, the results clearly suggest that the induced CD changes are primarily coming from electronic transitions of the metal complexes.

Because the large induced CD signals are primarily contributed from the metal complexes, and the complexes are achiral molecules that exhibit no intrinsic CD, the results further confirm the binding of the complexes to the nucleic acids.

An achiral molecule may acquire chirality in two different ways: (i) to bring the achiral molecule into close proximity to a chiral center, in other words, to bring an achiral chromophore into a chiral environment (e.g., phenol binding to cyclodextrin); or (ii) the achiral molecule may be arranged into a helical structure and thus acquire chirality (22, 23). Nucleic acids are well known to be inherently chiral because they contain a chiral sugar moiety. However, our results clearly show that the binding of the complexes to the nucleic acids does not always induce chirality. For example, neither the binding of complex **1** nor complex **2** to poly(dG)₂₅ gives any CD spectral changes, and the binding of complex **2** to poly(dC)₂₅ also gives rise to very small CD spectral changes. The results thus suggest that the chirality induced is not a simple consequence of bringing the platinum complexes close to the vicinity of the chiral nucleic acid, but rather more likely to be associated with the helical assembly of the platinum complexes upon binding to the anionic phosphate sites, induced by the propensity of these square-planar d⁸ platinum(II) terpyridyl units to stack via metal...metal and π ... π interactions. Supports for the helical supramolecular assembly of the complexes could be reflected by the mirror image relationship of the CD spectra obtained in the presence of poly(L-

[†]Assuming that the strong induced CD signals are from the metal complex, the calculated maximum and minimum $\Delta\epsilon$ values for poly(dT)₂₅ mixed with complex **1** would be 739 and -50 $M^{-1} cm^{-1}$, respectively.

glutamate) and poly(D-glutamate) (see above) and their similarities to the CD spectra in the presence of various oligonucleotides, which could also have different helix handedness. The results thus suggest that the metal complex cations upon binding to the oligonucleotides would self-assemble into a helical supramolecular assembly of different handedness. The magnitude of the induced CD intensity is also in the same range as other related helical systems (26, 27).

The differences observed in the new band formation, intensity variations of the UV-vis, emission, and CD spectra, and the preferred handedness of the helical self-assembly of the metal complex cations in the presence of the four different DNA homopolymers, namely poly(dT)₂₅, poly(dC)₂₅, poly(dA)₂₅, and poly(dG)₂₅, are apparently associated with the structural properties of the complexes, and more importantly, the primary and secondary structures of the oligonucleotides.

For poly(dT)₂₅, with which both complexes **1** and **2** show strong aggregation properties as revealed by the formation of the strong MMLCT bands in both the UV-vis and the emission spectra, the CD signal induced is suggestive of the self-assembly of the metal complexes into a helical array. On the other hand, poly(dA)₂₅, which gives much less pronounced UV-vis and emission spectral changes, with a very broad structureless emission spanning in the range of 500–850 nm, shows only a relatively small enhancement in the CD signal upon metal complex binding. Because poly(dT)₂₅ and poly(dA)₂₅ carry the same number of negative charges, there is no obvious reason to assume that the positively charged complex cations would interact electrostatically with poly(dA)₂₅ in a much weaker fashion than that with poly(dT)₂₅. The very broad emission is likely to be a mixture of MMLCT triplet emission which is a result of complex aggregation (at ≈800 nm) and complex monomer emission (17–21). Because poly(dA) has been shown to assume a helical structure (right-handed) stabilized through adenine base stacking interactions (25, 28, 29), it is likely that the hydrophobic interactions between the relatively hydrophobic planar adenine base and the square-planar platinum complex cations would reduce the tendency for the complex cations to stack with each other to form a helical self-assembly, resulting in the reduced chirality induced and the observation of emission from the mononuclear platinum species. On the other hand, for poly(dT), which does not have a helical structure and has little long range order and very little base stacking interactions between the thymine bases (28, 29), the relatively more hydrophilic structure together with the bulky methyl group of thymine would greatly reduce its hydrophobic interactions with the complex cations, thus favouring the self-assembly of the metal complexes to form helical assembly.

As mentioned earlier, when complexes **1** and **2** are mixed with poly(dC)₂₅ and poly(dG)₂₅, new UV-vis and emission bands of moderate intensity are observed (Fig. 2). The results clearly indicate that upon binding to the oligonucleotides, the metal complexes self-assemble through metal–metal and π – π stacking interactions, although to a lesser extent than that of the binding to poly(dT)₂₅. Although UV-vis and emission studies of the complexes mixed with poly(dC)₂₅ and poly(dG)₂₅ show similar extent of self-assembly, the CD experiments show quite dramatic difference. With poly(dC)₂₅, a CD spectrum in line with the helical assembly of the metal complex is observed for complex **1**, with opposite handedness to its self-assembly with poly(dT)₂₅, whereas for complex **2**, very small CD changes are observed, indicating no induced chirality and hence helicity of the complex self-assembly. Although poly(dC) is known to adopt a helical conformation under basic conditions (30), under acidic conditions, poly(dC) forms the very unique i-motif structure, as a result of the C–C⁺ (cytosine–protonated cytosine) base pairing [see supporting information (SI) Fig. 8 *Left*] (31–34). At pH 5.0, our results show improved self-assembly of complex **1** when

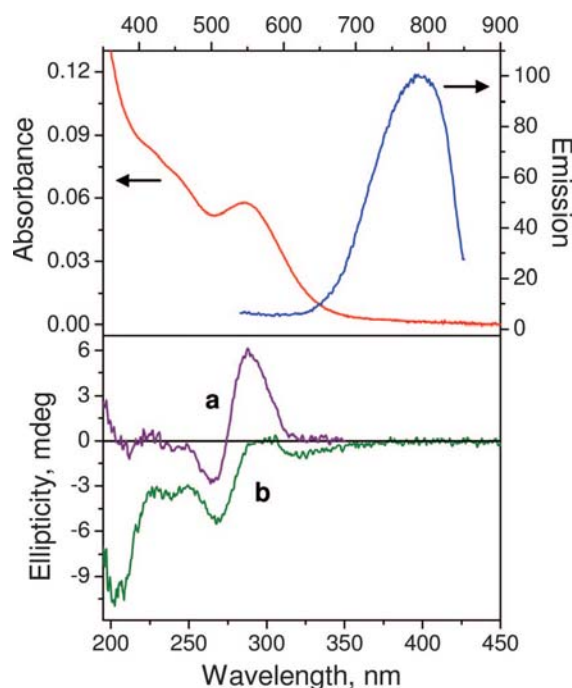


Fig. 5. Complex binding to i-motif. (*Upper*) UV-vis absorption and emission spectra of 90 μ M of poly(dC)₂₅ plus 30 μ M of complex **1**. (*Lower*) The corresponding CD spectra of poly(dC)₂₅ (line a), and its binding with complex **1** (line b). Medium used was 5 mM HOAc–NaOAc, 10 mM NaCl, pH 5.0.

mixed with poly(dC)₂₅, as revealed by the enhancement of the MMLCT bands in both the UV-vis and the emission spectra (Fig. 5 *Upper*). However, the CD spectrum induced by metal complex binding is found to be quite different from the helical assembly obtained previously, and also different from the CD signatures of the i-motif structure (Fig. 5 *Lower*), that is initially formed before metal complex binding. The very compact i-motif structure appears to facilitate the self-assembly of the complex cations, albeit different from the helical assembly as revealed by the CD.

With poly(dG)₂₅, no chirality is induced in either complex **1** or complex **2**. A CD signature of the parallel G-quadruplex structure is observed in the CD spectra with poly(dG)₂₅ (Fig. 3*IV*) (22, 35–39). The results suggest that the existence of poly(dG)₂₅ as the G-quadruplex structure, stabilized via hydrogen bonding interactions among four guanine bases (SI Fig. 8 *Right*), does not favor the formation of helical structures in the complex self-assembly process although aggregation via binding of the metal complexes to poly(dG)₂₅ does occur, as revealed by UV-vis and emission spectral changes. Throughout the current investigation, it is interesting to note that the more hydrophobic complex **2** usually gives weaker self-associated aggregates and lower degree of helical character in the organized assembly, presumably as a result of the stronger hydrophobic interactions with the nucleic acid bases. For poly(dC)₂₅, the different hydrophobic properties of these two complexes give the most dramatic difference, with the self-assembly of complex **1** giving a helical array, whereas that of complex **2** does not.

The oligonucleotide induced self-assembly of the metal complex is found to be chain length dependent. With a fixed molar ratio of complex **1** to nucleic acid base of 1:3, for poly(dT)₅, which contains only five monomer nucleotide units, UV-vis and emission studies show only very small intensity changes, indicating that the self-assembly is quite weak. For poly(dT)₁₀, UV-vis and emission studies indicate significant complex self-assembly, whereas for poly(dT)₁₅, the spectroscopic changes are

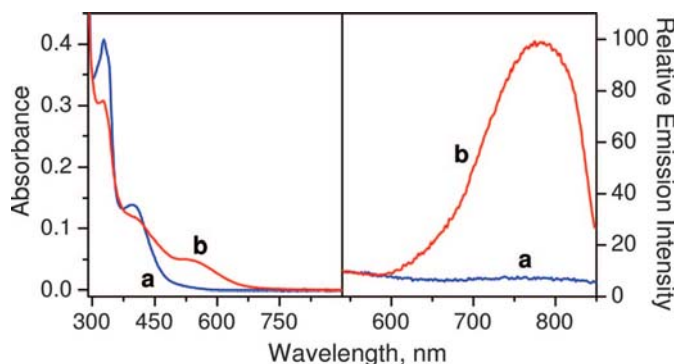


Fig. 6. UV-vis absorption (Left) and emission (Right) spectra of 30 μM of complex **1** (line a), and its binding to 90 μM of poly(dA)₂₅ (line b). Medium used was 80% aqueous buffer (5 mM Tris-HCl/10 mM NaCl, pH 7.5) plus 20% CH₃CN.

comparable to those with poly(dT)₂₅, indicating strong complex helical self-assembly. We also tested poly(dT)₂₀ and poly(dT)₅₀, and both give results similar to poly(dT)₂₅.

The effect of addition of an organic solvent to the complex self-assembly properties is also studied, because it is well known that addition of an organic solvent could reduce the hydrophobic interactions in an aqueous medium (40, 41), and in this case reduce the $\pi\cdots\pi$ interactions with the oligo that compete with the formation of complex self-assembly. In the binding studies of complex **1** with poly(dA)₂₅, addition of 20% CH₃CN completely eliminates the complex monomer emission, and the UV-vis and emission spectral changes also indicate rather good self-assembly of the complex (Fig. 6). The results show that the increase in organic solvent content reduces the $\pi\cdots\pi$ hydrophobic stacking interactions between the metal complex and the nucleic acid base that give rise to complex monomer emission. However, weaker CD signals are observed, indicating a reduced helicity under such conditions. Addition of 20% trifluoroethanol (TFE) also gives similar results. With poly(dT)₂₅, in the presence of 20% TFE, complex **1** gives nicely defined helical assembly, as revealed from the UV-vis, emission and CD measurements, albeit a bit less strongly than in aqueous buffer.

An oligonucleotide sequence containing all four bases was randomly selected. The sequence is CAT TAC TGG ATC TAT CAA CAG GAG (a standard vector primer). In pure buffer, its mixture with complex **1** shows only weakly induced self-assembly of the complex, presumably due to the hydrophobic interactions between the nucleic acid bases and the complex cation, and possibly some ill-defined secondary structures of the oligonucleotide. However, when 20% TFE was added, both the UV-vis and emission spectra indicate good self-assembly of the metal complex, and a strong CD signal, indicative of an assembly that is strongly helical in nature, is observed (SI Fig. 9).

Based on the observation of different trends with the four different nucleobases, it seems that the subtle competition between the hydrophobic π stacking interactions with DNA and the metal-metal interaction assisted self-assembly of the platinum(II) complexes plays an important role in governing the delicate balance between them.

We have also extended our studies to double-stranded DNA. Preliminary studies show that upon addition of 30 μM of complex **1** to a poly(dA)₂₅-poly(dT)₂₅ duplex, prepared by mixing equal amounts (45 μM base concentration) of the respective poly(dA)₂₅ and poly(dT)₂₅, both the UV-vis and emission spectra show pronounced MMLCT bands, and changes in the CD signal typical of the complex helical assembly are also observed. With an increasing amount of the duplex DNA, the MMLCT absorption band gradually disappears, and a new band at 425 nm emerges in the UV-vis spectra (Fig. 7 Left). Concom-

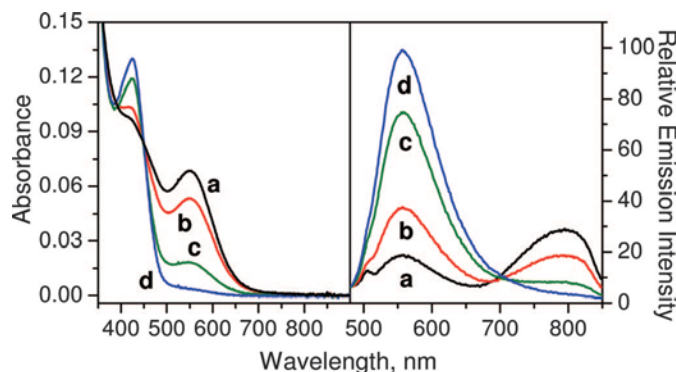


Fig. 7. UV-vis absorption (Left) and emission (Right) spectra of 30 μM of complex **1** binding to 45 μM of poly(dA)₂₅ plus 45 μM of poly(dT)₂₅ (line a); 90 μM of poly(dA)₂₅ plus 90 μM of poly(dT)₂₅ (line b); 180 μM of poly(dA)₂₅ plus 180 μM of poly(dT)₂₅ (line c); and 270 μM of poly(dA)₂₅ plus 270 μM of poly(dT)₂₅ (line d). Medium used was 5 mM Tris-HCl/10 mM NaCl, pH 7.5.

itant with the UV-vis spectral changes, the emission band at ≈ 800 nm gradually disappears, and a new strong emission band at ≈ 557 nm emerges (Fig. 7 Right). In addition, at high duplex concentration [270 μM poly(dA)₂₅ plus 270 μM poly(dT)₂₅, base concentration], addition of 30 μM of complex **1** causes very small CD spectral changes. Because the square planar platinum terpyridyl type complexes are well known duplex DNA intercalators (11–13), the results suggest that, at low duplex concentrations, most of the complexes are bound to the anionic phosphate groups on the DNA via electrostatic interactions, leading to a helical self-assembly, whereas at high concentrations of the duplex DNA, the majority of the complexes would intercalate into the duplex, and therefore self-assembly of the complexes is not observed (11–13, 16). The shift of the absorption and emission bands to a shorter wavelength of 425 and 557 nm, respectively, strongly indicates that the complex cations are in a very different environment, presumably stacked between the nucleotide base pairs in an intercalative manner.

Summary and Prospects

A general label-free method for optical sensing and characterization of single-stranded nucleic acid has been demonstrated. We show that binding of the alkynylplatinum(II) terpyridyl complexes to single-stranded nucleic acid via electrostatic interactions induces aggregation of the complex molecules. The planar complex molecules subsequently form self-assembly via metal-metal and $\pi\cdots\pi$ interactions. As a result, remarkable UV-vis, emission, and CD spectral changes are observed.

The results clearly show that the spectroscopic property changes are associated with the primary and secondary structures of the nucleic acid. In some cases the complex self-assemblies are strongly helical, and in others they are not. The degree of the complex self-assembly, i.e., the intensity of the MMLCT absorption, MMLCT triplet emission, and also the degree of the CD signal induced of the complex self-assembly, are different depending on the particular nucleic acid studied.

The formation of new UV-vis and emission bands also suggests better detection contrast and sensitivity. More importantly, the strong spectroscopic property changes, especially the CD spectral changes, indicate that the present method could possibly be used to study the secondary structure and structure/conformation changes of the nucleic acid. It is possible that the spectroscopic properties could be finely tuned by the selection of other suitable metals that are capable of metal-metal interactions, and other coordinating ligands of various properties, and a detailed understanding of the complex assemblies (e.g., the length of the assembly, dimer, trimer, or possibly longer oli-

gomers, etc.). We envisage that the present approach may facilitate nucleic acid-related biological research.

Materials and Methods

General Details. Oligonucleotides were obtained from Sigma-Proligo (St. Louis, MO). Throughout the current investigation, nucleic acid concentration was expressed as the total concentration of the nucleic acid base; unless otherwise specified, a buffer solution containing 5 mM Tris·HCl and 10 mM NaCl (pH 7.5) at ambient temperature was used. Poly(L-glutamic acid), poly(D-glutamic acid), and poly(L-aspartic acid) in their sodium salt form were purchased from Sigma.

UV-vis absorption spectra were recorded on a Cary 50 spectrophotometer (Varian, Palo Alto, CA) equipped with a xenon flash lamp. Steady-state excitation and emission spectra were obtained by using a Spex Fluorolog-2 Model F111 spectrofluorometer. The emission spectra were collected at an excitation wavelength of 430 nm and were not corrected for PMT response. Microquartz cuvettes with 10-mm path length and

2-mm window width were used for UV-vis and emission measurements. CD measurements were performed on a Jasco (Tokyo, Japan) 720 spectropolarimeter using a cylindrical quartz CD cell with 1-mm path length. Details of instrumentation are given in *SI Text*.

Metal Complex Synthesis. Complex **1** was synthesized by modification of a literature procedure for complex **2** (20). The details of the synthesis and characterization are given in *SI Text* and SI Scheme 1.

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